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Measuring Alcohol-Induced Secondary Structure Changes of SNAP-25A

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Abstract

Alcohol has been consumed by humans for thousands of years and has a known inhibitory effect on neurotransmission. Here we explore the effect of ethanol on the folding of SNARE proteins known to drive neurotransmitter release (exocytosis) in neurons. The SNARE proteins SNAP-25, syntaxin, and VAMP provide the four helical regions (SNARE domains) that form a coiled-coil complex required for exocytosis. This complex is continually formed and unwound as exocytotic vesicles fuse and are recycled.

Circular Dichroism was used to measure secondary structure of SNAP-25's first SNARE domain SN1. We observed an increase in α-helical structure followed by precipitation as a ß-sheet when ethanol (EtOH) is added. This is similar to the helical shift observed when SNAP-25 forms a complex with syntaxin and VAMP. These data show that ethanol may induce some of its effects by altering the SNARE fusion machine, consequently playing a role in decreasing neurotransmitter release.

Introduction

Circular Dichroism Spectroscopy (CD) is a form of spectroscopy that is based on the different absorptions of left- and right-handed circularly polarized light. It can be used to predict the secondary structure of proteins because α-helices, ß-sheets, and random coils of protein produce different absorption spectrums and has previously been used to study SNARE proteins [3].

We used CD to measure conformational changes of SNAP-25A's first SNARE domain SN1. SNAP-25 provides two of four helical regions (SN1 and SN2) that coil together with VAMP and syntaxin to pull exocytotic vesicles close enough to the cell membrane for fusion to occur, facilitating neurotransmission. Disfunction of SNAP-25 *in vivo* has been linked to many diseases, including ADHD, Alzheimer's, and insulin resistance [1].

Working Hypothesis:

***In vivo*, neurotransmission is influenced by SNAP-25's secondary structure, which is affected by alcohols, such as ethanol. Examining how these properties respond to environmental changes should reveal how they modulate SNARE complex formation and neuro-transmitter release at the synapse.**

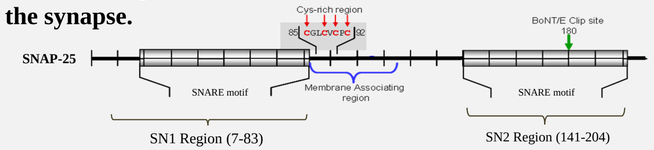


FIGURE 1: SNAP-25 has 2 SNARE domains that tend to form α-helixes and a linker region. The SN1 region's ethanol-induced conformational changes were studied.

Ethanol induces changes in secondary structure of SNAP-25A

Using Circular Dichroism (CD) spectroscopy, we detected significant changes in the secondary structure of the SN1 and SN2 domain of SNAP-25 (see Fig. 1). Following data collection, three methods were used for secondary structure analysis of SN1 (see Fig. 3,4,5).

FIGURE 2: Data. Addition of ethanol altered SN1 from primarily random coil to primarily alpha helix. We scanned at 0% ethanol (solid **black** line), then added ethanol up to 50% volume (solid red line). The sample was evaporated and 100% ethanol added (gray dotted line). Sample was diluted to 50% ethanol (red dashed line). Decreased protein solubility was seen above 50% ethanol. Note that the concentration of the protein changes at each progressive spectra due both to precipitation (ppt) and addition of EtOH.

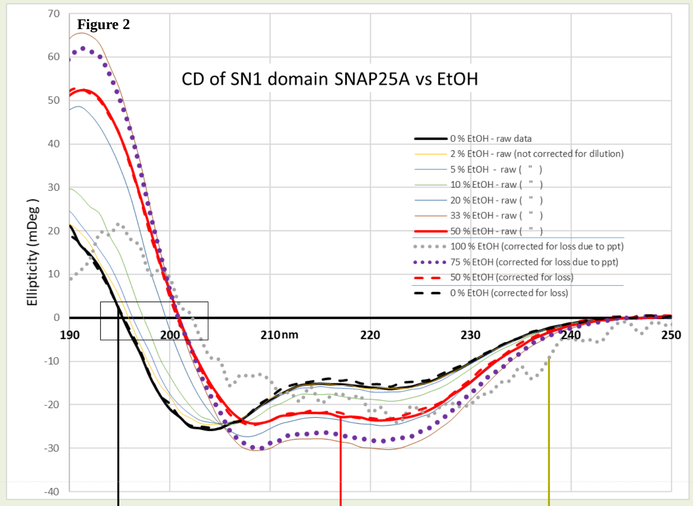


FIGURE 3: Method 1. CD spectra were fit using the "DichroWeb" (based on the estimated protein conc.) [2]. Shown (L to R) are the CD spectra of SN1 with 0%, 50%, and 100% EtOH. Each spectra lists the calculated secondary structure.

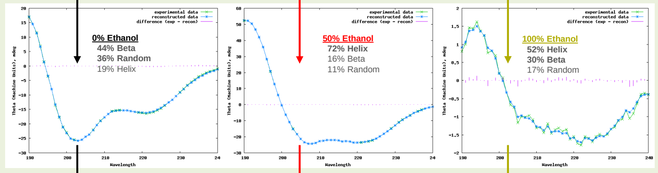
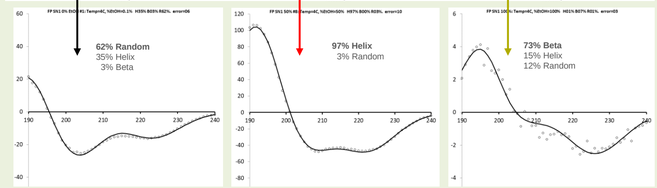


FIGURE 4: Method 2. Beta-Corrected Fit. Previously, we have observed that at pH 5, SNAP25B folds nearly 100% into an unusual beta sheet with a negative peak at 227nm rather than the usual 218nm. This spectra is again seen at 100% EtOH. Shown are the refit spectra using this correction. **Note that at 0% EtOH, SN1 exists primarily as random coil; at 50% EtOH, as alpha helix; and at 100% EtOH, as beta sheet** (after correction for ppt).



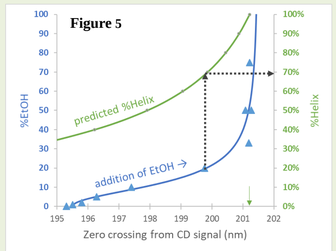


FIGURE 5: Method 3. We noted that the zero crossings of the spectra (black box in Fig. 2) shifted with EtOH addition (**blue** curve). We used our beta-corrected fitting method to predict how this zero crossing correlates with helicity of SN1 (**green** curve). This allows us to easily convert the zero crossing into percent helix (e.g. at 20% EtOH, CD crosses zero at 199.8nm, corresponding to ~70% Helix, dotted arrows). **The zero-crossing method allowed us to calculate %Helix independent of concentration.**

CD Method: Protein was suspended in 20 mM Potassium Phosphate buffer (pH 7.6) at a concentration of ~160µg/mL. Each CD spectra was fit using the "DichroWeb" CDSSTR algorithm [2] with *Helix=Helix1+Helix2, Beta=Strand1+Strand2+Turns, Random=Unordered.* Measurements were taken between 260 - 185 nm, at 5 seconds per nm. Between scans, ethanol was added to the sample and mixed, leading to an expected sample dilution.

Comparison and Conclusions

FIGURE 6: Comparison of Methods. For every EtOH dose, the percent Helix is plotted as calculated from DichroWeb [2] (blue), the Beta-corrected Fit (orange), or the zero crossing (green). Only the zero crossing method is concentration independent.

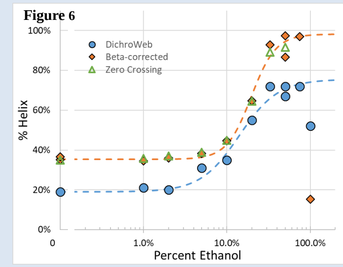
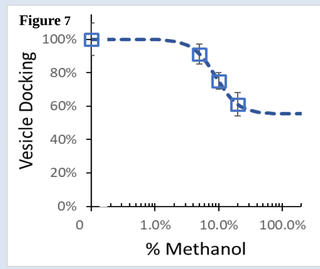


FIGURE 7: Relevance. SNARE-SNARE-mediated vesicle docking was measured as previously reviewed [4]. The decrease in docking with alcohol may be due to helix formation which shortens the SNARE domain and hence decreases probability of SNARE-SNARE interaction and exocytosis.



Conclusions:

1. **SNAP-25 appears to be an intrinsically disordered protein with the ability to fold as alpha helix, beta sheet, or random coil depending on environmental parameters.**
2. **Zero crossing is a simple method to estimate percent helix when a predominantly helical protein is forming or melting and is independent of protein concentration.**
3. **The extent of helicity of the SNARE motif in SNAP-25 may determine probability of SNARE proteins docking at the synapse, thus regulating neurotransmission in the brain.**

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